



A Comparison of Performance Using Three Bioreactor Systems

CelliGen BLU Single-Use Bioreactor vs. An Autoclavable Bioreactor and Rocker/Bag Bioreactor

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Abstract

New Brunswick Scientific (NBS) has introduced a new benchtop cell culture bioreactor that combines the benefits of single-use technology with the trusted performance and true scalability of a stirred-tank design. CelliGen™ BLU eliminates the need for autoclaving and cleaning, reduces validation requirements, provides rapid turn-around between runs, minimizes startup costs, and significantly reduces the risk of contamination because the culture vessel is only used once and then discarded. A compact controller enables advanced process management for research or cGMP manufacturing. This paper compares performance of the CelliGen BLU vs. New Brunswick's autoclavable CelliGen 310 and a competitor's rocker and bag-style bioreactor.

Single-Use, Stirred-Tank Vessels

CelliGen BLU features interchangeable, single-use, rigid-wall, stirred-tank vessels in 5.0 and 14.0 L total volume capacities. Vessels are delivered pre-assembled with pitched-blade impeller, porous microsparge, and all the necessary tubing, filters, and connectors; and come pre-sterilized, ready for use right out of the box. All components in product contact are made of materials that meet USP Class VI standards and have been tested for leachables and extractables, making these vessels appropriate for cGMP environments.

Headplate penetrations are provided for RTD temperature sensor, pH and DO probes, three addition ports, harvest, exhaust, sample, overlay, and gas sparge. A reusable heat blanket with window cut-out for viewing your cultures is provided for maintaining temperature in the culture vessel. DO is monitored via a non-invasive, reusable polarographic DO probe. pH is monitored via a non-invasive optical pH probe and disposable fluorescence sensor.

Advanced Process Control

CelliGen BLU's control station includes NBS' powerful Reactor Process Controller (RPC) firmware to provide advanced process management and monitoring capability. An adjustable-position, 15" industrial color touchscreen monitor makes it easy to set and monitor up to 32 process parameters, store up to 10 recipes, as well as view up to 8 parameters simultaneously on a user-customizable trend graph. Built-in security features include variable levels of control functionality for operators, supervisors and administrators, and passwords to prevent unauthorized users from making any changes to the system.

The compact control station includes three high-performance, assignable, fixed-speed pumps for additions and harvesting. Built-in controls are also provided for temperature, pH, dissolved oxygen (DO) and 3- or 4-gas mixing. Up to four Thermal Mass Flow Controllers (TMFC) for fully independent gas control are available for sparging, and a gas overlay with TMFC or Rotameter is available for independent control of gas entering the vessel headspace. An optional weight scale for measuring additions and level, as well as optional gas analyzers, BioCommand® supervisory control and data acquisition (SCADA) software, validation packages and more, enable customization to your needs.



Comparison to Similar Culture Systems

To prove the CelliGen BLU's ability to control growth conditions at setpoint as well as to produce high cell densities, researchers at New Brunswick Scientific's in-house laboratory compared growth of a Chinese Hamster Ovary (CHO) cell line using three bioreactor systems:

- The CelliGen BLU benchtop bioreactor with 5 liter pre-sterilized single-use vessels
- The CelliGen 310 benchtop bioreactor with 5 liter autoclavable vessels (NBS)
- A bag and rocker style culture system with 10 liter single-use bags.

The CHO line (ATCC, Manassas VA) was pre-adapted to use in a serum-free medium, and a CD CHO serum-free medium (Invitrogen Cat # 12490-025) was used.

Control parameters for all three systems, shown below, were set as identically as possible.

CelliGen BLU Setpoints:

- Temperature 37°C
- pH set point 7.0
- Dead-band 0.10
- DO 40%
- Agitation 70-80 rpm
- Gas overlay at 0.2 - 0.3 standard liters per minute (SLPM) via 4-Gas mode for the entire run
- Gas sparger using 4-Gas mode at 0-20 CCM via Cascade control from the Dissolved Oxygen (DO) to Gasflo, after 2 days of the run.

CelliGen 310 Setpoints were identical, except:

- Agitation 80-100 rpm
- Gas overlay at 0.2 - 0.3 SLPM using 4-Gas mode for the first 2 days
- Gas sparger at 0.1 - 0.20 SLPM by 4-Gas mode after 2 days of the run

Bag & Rocker Bioreactor Setpoints are thought to be nearly equivalent to those used in the stirred-tank reactors:

- Temperature 37°C
- pH about 7.0
- DO about 40%
- Rocking speed 18 rpm
- Rocking angle 8°
- Gas overlay at 0.1 - 0.3 SLPM by air with 0 - 5% CO₂ based on the pH

Each of the batch runs lasted 7 days. Daily off-line measurements of glucose and lactate concentration were read using a YSI 2700 analyzer (YSI Inc., Yellow Springs OH), and cell density and cell viability were measured using a NucleoCounter (NBS).

The CHO cells in all three bioreactor systems grew steadily through day 5 of the 7-day run (**Table 1**), coinciding with availability of nutrients in the medium. By day 5, a nutrient source, glucose, had become nearly depleted from the medium (**Fig. 2**). Cell viability in all three systems also remained high, at 96% or better, through day 5. The maximum viable cell count attained in each system was as follows: 5.55×10^6 cells mL⁻¹ by the CelliGen BLU compared to 5.39×10^6 cells mL⁻¹ for the autoclavable system, and 4.77×10^6 cells mL⁻¹ for the bag-and-rocker system.

Day	CelliGen BLU bioreactor			CelliGen 310 bioreactor			Bag-and-rocker system		
	Total cells (x10 ⁶ ml ⁻¹)	Viable cells (x10 ⁶ ml ⁻¹)	Viability (%)	Total cells (x10 ⁶ ml ⁻¹)	Viable cells (x10 ⁶ ml ⁻¹)	Viability (%)	Total cells (x10 ⁶ ml ⁻¹)	Viable cells (x10 ⁶ ml ⁻¹)	Viability (%)
0	0.31	0.30	97.9	0.31	0.30	97.9	0.31	0.30	97.9
1	0.69	0.68	97.1	0.64	0.62	96.5	0.61	0.58	96.8
2	1.42	1.39	97.6	1.31	1.29	97.9	1.33	1.30	97.6
3	2.57	2.51	97.6	2.47	2.41	97.8	2.36	2.32	98.4
4	4.02	3.92	97.5	4.04	3.98	98.6	3.89	3.83	98.5
5	5.70	5.55	97.3	5.46	5.39	98.7	4.83	4.77	98.9
6	5.98	4.52	76.6	5.67	4.13	72.7	5.46	3.67	67.3
7	6.71	3.21	47.8	6.25	3.12	49.7	5.59	2.73	48.8

Table 1. Comparison of CHO growth and viability achieved using three different bioreactor systems.



Figure 1. The new CelliGen BLU cell culture bioreactor features single-use, stirred-tank vessels and an advanced process controller for growth of animal cells in cGMP-compliant research and production environments.

Conclusion

While no effort was made to optimize either the medium or the cell culture process control parameters in any study, this data demonstrates that the CelliGen BLU bioreactor is an efficient system for the culture of CHO cells, and can outperform bag-style systems. For a complete protocol on system setup, or for additional information on the CelliGen BLU, see www.nbsc.com/BLU.

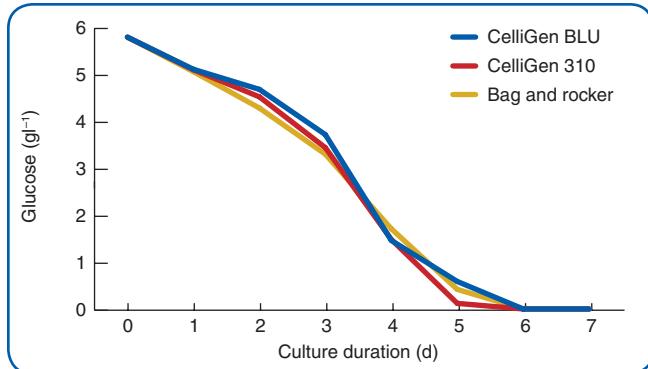


Figure 2. Glucose concentration versus culture time for CHO cell cultures in three benchtop bioreactor systems.

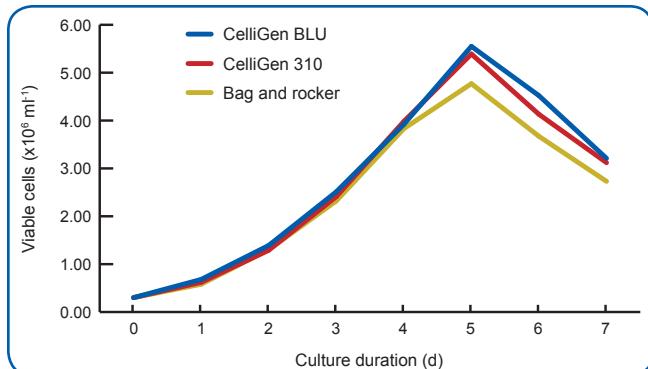


Figure 3. Viable cell count peaked at 5.55×10^6 cells mL^{-1} in the CelliGen BLU, compared to 5.39×10^6 cells mL^{-1} in the CelliGen 310 autoclavable system and 4.77×10^6 cells mL^{-1} in the bag-and-rocker system.

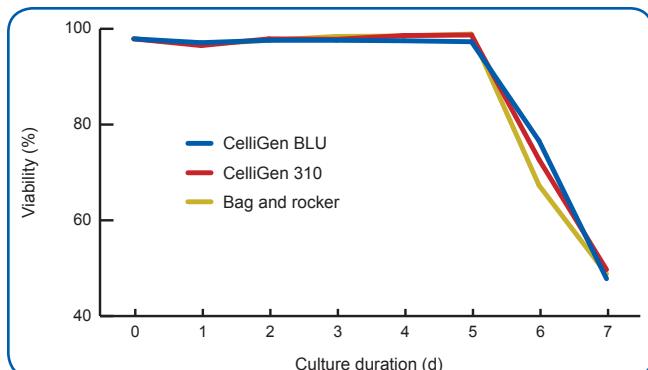


Figure 4. Cell viability in all three systems was extremely high through day 5, until the nutrient source in the batch run became depleted.

CelliGen™ BLU

*Benchtop Cell Culture System
5.0 & 14.0 Liter Single-Use, Stirred-Tank Bioreactor*



New Brunswick
an eppendorf company

The CelliGen™ BLU Bioreactor: Proven Stirred-Tank

New Brunswick Scientific has introduced a new benchtop cell culture bioreactor that combines single-use technology with the trusted performance and true scalability of a stirred-tank design.

CelliGen BLU has been engineered for high-density animal cell culture in research or production, using interchangeable, single-use, stirred-tank vessels in 5.0 and 14.0 L total volume capacities. A compact controller enables advanced process management for research or cGMP manufacturing.

Key Benefits

- The single-use vessel eliminates autoclaving and cleaning, provides rapid turn-around between runs, reduces risk of contamination, reduces validation requirements and minimizes initial startup costs
- The integrated control station enables advanced gas management and process control
- Stirred-tank design provides proven performance and scalability
- Unique pH and DO technology makes probe insertion totally noninvasive and autoclaving unnecessary

Single-Use Vessel, Stirred-Tank Design

Interchangeable, rigid-wall, 5 and 14 liter stirred-tank vessels come pre-sterilized and ready for use — no cleaning, autoclaving, or assembly needed. All vessel components in product contact are made of USP Class VI materials and have been tested for leachables and extractables, making them appropriate for GMP environments.

Vessels Include:

- Bi-directional, pitched-blade impeller
- Microsparger
- pH sensor
- Tubing (silicone and C-Flex®, for use with tube welders)
- 0.2 µm Filters
- Sealed magnetic drive



14.0 Liter Vessel

5.0 Liter Vessel

Ports and Penetrations:

- RTD/temperature
- Noninvasive optical pH
- Noninvasive polarographic DO
- Additions (3)
- Harvest
- Overlay
- Sparge
- Sampling
- Exhaust line with heater

Single-use, stirred-tank vessels are delivered pre-sterilized and pre-assembled, ready for use right out of the box



Ideal for research or production environments, CelliGen BLU can be custom-configured to meet your process needs.

Compact Control Station

New Brunswick's powerful RPC (Reactor Process Control) firmware provides integrated control of up to 32 process parameters, and includes time-saving recipe features, built-in security and ability to simultaneously monitor up to eight parameters on user-customized trend graphs.

Sophisticated Control Station includes:

- Industrial touchscreen interface with adjustable position, 15" (38 cm) color display
- 3 integrated pumps (with option to add more)
- Magnetic-drive motor
- Control of temperature, pH, DO and 3- or 4-gas mixing
- 7 analog inputs and 7 analog outputs for addition of ancillary devices
- Media bag hanger

Gas Flow Control Options

- 1, 3 or 4 Thermal Mass Flow Controllers (TMFC) or a Rotameter for sparge gas
- Rotameter or TMFC for optional gas overlay

Additional Options

- Scale for measuring additions and harvest
- Validation packages
- Gas regulators
- Media/supplement addition kits
- BioCommand® SCADA software

Design Meets Single-Use Technology



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Compact, Ergonomic Control Station saves valuable lab space

Easy-to-Access Utility Connections enable quick setup

Advanced Gas Control:

- Built-in controls enable 3- or 4-gas mixing
- Choose 1, 3 or 4 TMFCs for automatic control of DO & pH. Manual Rotameter also available
- 4-gas overlay with choice of TMFC or Rotameter can also be added

Customizable PI Values allow fine-tuning your process

Three Built-in, High-Performance, Assignable, Pumps simplify additions and harvest. One 14 rpm & two 109 rpm, provided. Option to front-mount additional pump heads

Scale Option lets you monitor and control vessel volume or feeds



Magnetic-Drive Motor (25 - 200 rpm) with fully enclosed bearings which eliminate contamination risk

Pitched-Blade Impeller, with upflow or downflow selectable via touchscreen

Microsparge (7 - 12 µm) provides high mass transfer at low flow rates

Headplate Tubing and Connections

Use	Tubing Type	Length	Size	Connection/Filter
Addition 1	C-Flex®	20" (500 mm)	1/8" ID x 1/4" OD (3.18 mm x 6.4 mm)	1/8" Luer-Lok connect
Addition 2	C-Flex	28" (700 mm)	1/4" ID x 3/8" OD (6.4 mm x 9.6 mm)	1/4" CPC quick-connect
Addition 3	C-Flex	28" (700 mm)	1/4" ID x 3/8" OD (6.4 mm x 9.6 mm)	1/4" CPC quick-connect
Sample	C-Flex	20" (500 mm)	1/8" ID x 1/4" OD (3.18 mm x 6.4 mm)	CLAVE® NeedleFree connector
Harvest	C-Flex	28" (700 mm)	1/4" ID x 3/8" OD (6.4 mm x 9.6 mm)	1/4" CPC quick-connect
Exhaust	Silicone (platinum-cured)	8" (200 mm)	3/8" ID x 1/2" OD (9.5 mm x 12.7 mm)	0.2 µm filter
Sparge	Silicone (platinum-cured)	2" (50 mm)	1/16" ID x 1/8" OD (1.59 mm x 3.18 mm)	0.2 µm filter
Overlay	Silicone (platinum-cured)	2" (50 mm)	1/16" ID x 1/8" OD (1.59 mm x 3.18 mm)	0.2 µm filter

Large, Color Touchscreen Interface lets you easily enter, monitor and control process parameters

Other Advantages, not shown in this image:

Innovative pH Sensor and Proprietary DO Probe Sleeve Design:

- Eliminate contamination risk, as there is no direct contact between probes and vessel contents
- Save hours of prep time and labor, as no autoclaving or cleaning is needed; and the reusable DO probe can be left attached to the control station, polarized for ready use
- Enable vessel setup right on the bench – no biosafety cabinet / hood needed for operation.
- Extend DO probe life, as it is never autoclaved

Vessel Heat Blanket provides uniform temperature control

Exhaust Line Heat Blanket prevents filter clogging



Separate harvest and sample lines enable use as a continuous perfusion system

CelliGen™ BLU Bioreactor Specifications¹

Single-Use Cell Culture Vessel	Total Volume	5.0 Liters	14.0 Liters
	Working Volume	3.75 Liters	10.5 Liters
	Minimum Volume	1.25 Liters	3.5 Liters
Controller	Control Station	Controls up to 32 control loops; stores 10 recipes and 8 process variables for trend graphing. Includes an industrial touchscreen monitor/user interface, 3 built-in pumps and connectors for all utilities and communications signals	
	Touchscreen Interface/Display	15-inch (38.1 cm) color industrial monitor with adjustable-angle swivel	
Temperature	Indication	Digital display in 0.1°C increments	
	Range	From 5°C above ambient temperature to 40°C	
	Control	P&I for heating using a Heat Blanket (cooling via ambient environment)	
	Sensor	Platinum RTD probe	
Agitation	Drive	Permanent magnet motor with high torque input	
	Indication	Digital display in 1 rpm increments	
	Range	25 - 200 rpm	
	Control	PI-controlled	
	Impellers	Pitched blade (upflow or downflow selectable via touchscreen)	
Exhaust	Filter	0.2 µm disposable filter	
	Tube	Heat blanketed	
Aeration	3- or 4-Gas System	Up to 4 gases, including air, N ₂ , CO ₂ and O ₂ , delivered to sparge and overlay	
	Flow Control	Automatic or manual options	
	Sparge	1, 3 or 4 TMFCs (0.002 - 0.1 SLPM) – Automatic option 1 Rotameter (0.001 - 0.1 SLPM) – Manual option	
	Optional Overlay	1 TMFC (0.1 - 3.0 SLPM) – Automatic option 1 Rotameter (0.1 - 3.3 SLPM) – Manual option	
	Sparge Element	Porous microsparger (6 - 12 µm)	
	Inlet Filters	0.2 µm disposable filter	
	Indication	Digital display in 0.01 pH increments	
pH	Range	6 - 8 pH	
	Control	P&I	
	Probe	Noninvasive optical pH probe. Single-use fluorescence sensor is included with each vessel	
DO	Indication	Digital display in 0.1% increments	
	Range	0 - 200%	
	Control	P&I	
	Probe	Noninvasive, reusable polarographic probe	
Pumps	Pump 1	Assignable high-performance peristaltic pump Fixed speed (14 rpm) or variable-duty cycle Available control modes: Off, On, Prime	
	Pumps 2 and 3	Assignable high-performance peristaltic pumps Fixed speed (109 rpm) or variable-duty cycle Available control modes: Off, On, Prime	
Utilities	Gas	5 - 6 PSIG maximum	
Electrical Requirements	100 - 120 VAC	50/60 Hz, Single phase, 15 Amps	
	200 - 240 VAC	50/60 Hz, Single phase, 15 Amps	
Net Weight	Control Station	88 lbs. (40 kg) with touchscreen	
	Touchscreen	15 lbs. (6.8 kg)	
	Vessel empty without motor²	5.0 liter vessel: 2.9 lbs. (1.3 kg)	14.0 liter vessel: 3.9 lbs. (1.8 kg)
Overall Dimensions (W x D x H)	Control Station & Vessel	25 x 24 x 34 inches (63 x 61 x 86 cm), including touchscreen	
Input/Output Connections and Comm Ports	Analog Inputs/Outputs	Up to 7 analog inputs and up to 7 analog outputs to add auxiliary equipment, usable as follows: <ul style="list-style-type: none">• 3 inputs and 3 outputs – interchangeable 0 – 5 V or 4 – 20 mA• Up to 4 additional 0 – 5 V inputs and 4 additional 0 – 5 V outputs available, depending on TMFC configuration	
	2 USB Ports	Import firmware/software upgrades and export trend data Connect optional 8-port serial box for scales, etc.	
	Communications Port	For optional BioCommand®/SCADA software	
Ambient Operating Conditions		10 - 30°C up to 80% relative humidity, non-condensing	
Regulatory Compliance		 	CAN/CSA-C22.2 Nos. 1010.1 & 1010.2.010 UL Standard UL-61010A-1 & 61010A-2-010

1. Specifications subject to change without notice. 2. Vessel weight does not include probes, exhaust heater or other options.

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